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Impact of Familial Hypertrophic Cardiomyopathy-Linked Mutations in the N-Terminus of the RLC on β-Myosin Cross-Bridge Mechanics
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Familial Hypertrophic Cardiomyopathy (FHC) is associated with mutations in sarcomeric proteins of which myosin regulatory light chain (RLC) is included. Here we studied the impact of the N-terminal FHC mutations on the molecular mechanism of β-Myosin heavy chain cross-bridge mechanics using the in vitro motility assay. To generate mutant β-Myosin, native pig RLC was depleted from porcine cardiac myosin heavy chain and reconstituted with mutant (A13T, F18L & E22K) or wild-type human RLC. We characterized the mutant myosin force and motion generation capability in the presence of a frictional load and thin filament regulatory proteins. All three mutants exhibited reductions in maximal filament velocity when tested with both calcium-regulated (40.5% ± 1.07, 28.9% ± 1.11, & 24.5% ± 1.14 A13T, F18L & E22K resp.) and unregulated filament (34.4% ± 0.8, 19.1% ± 0.9 & 5.6% ± 0.9 resp.) compared to wild-type. Furthermore, all three mutants displayed reductions in calcium sensitivity (pCa 50) (~5.13%, ~6.52% & ~4.50% resp.) as well as cooperative activation (~22%, ~42% & ~34% resp.) of regulated velocity. These results suggest that the known FHC mutants on the N-terminus of the RLC affect formation of strong binding cross-bridges thereby reducing maximal force production. This work is supported by grants to the following people HL077280 (JM) HL071778 (D S-C).

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The Effect of Regulatory Light Chain Phosphorylation on Myosin Bearing Familial Hypertrophic Cardiomyopathy-Linked Mutations
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Familial Hypertrophic Cardiomyopathy (FHC) is characterized by hypertrophy of the left ventricle that can often be preceded by diastolic dysfunction. The clinical presentation of the disease varies widely from asymptomatic to progressive heart failure to sudden cardiac death. FHC is caused by mutations in 1 of the 14 genes that encode for sarcomeric proteins. Two FHC mutations, N47K and R58Q, located in the regulatory light chain (RLC) of myosin have previously been shown to reduce actin filament velocity under load, stemming from a more compliant lever arm (Greenberg et al., PNAS add details 2010). The RLC mechanically stabilizes the neck/lever arm of myosin, which is crucial to myosin's ability to transmit contractile force. Phosphorylation of the RLC can impart stiffness to the myosin lever arm. In the work presented here, *in vitro* motility assays are utilized to investigate the effects of RLC phosphorylation on the FHC RLC mutant phenotype in the presence of an α-actinin frictional load. When co-incubated with a 0.5 μg/ml α-actinin frictional load, the R58Q mutation reduces actin sliding velocity to 54% of WT values, consistent with previous findings that myosin bearing the R58Q mutation exhibits reduced force production. Phosphorylation of R58Q mutant myosin restored velocity to 68% of WT velocity. These results suggest that that RLC phosphorylation may have a minor inhibitory effect on the FHC phenotype at the molecular level. Supported by AHA- 12PRE11910009 (AK), 10POST3420009 (PM), NIH- HL071778 & HL090786 (DSC) and HL077280 (JM).

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Effects of Hcm-Linked Mutations in Myosin Essential Light Chain and the Role of Serine-195 Pseudo-Phosphorylation
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In this study we investigated whether phosphorylation of myosin ELC at Serine 195, identified by proteomic analysis as a novel phosphorylation site, and shown to affect contractility in zebrafish cardiomyocytes, can play a role in cardiac muscle contraction. We also investigated whether S195D pseudo phosphorylation of ELC could rescue an ELC induced hypertrophic cardiomyopathy (HCM) phenotype. To test this, we exchanged human recombinant WT-ELC, WT-S195D and ELC-A57G, E143K and M173V mutants in skinned porcine papillary muscle fibers and porcine cardiac myosin, both expressing the β-myosin heavy chain (MHC). In myosin exchange experiments, we observed a stronger binding of WT-S195D to MHC (lower Kd), but the degree of ELC exchange vs. WT was significantly lower (~48% vs. ~69%). However, no changes in %-reconstitution were observed among all recombinant ELC mutants when compared to WT. In the ELC-reconstituted fibers, WT-S195D was seen to exchange with a significantly lower efficiency compared with WT (~33% vs 47%), but it also caused a small increase in maximal force (by ~3 kN/m2). An increase in maximal tension upon pseudo-phosphorylation of ELC was in accord with the MLCK-induced or pseudo (with S15D mutation)-phosphorylation observed for the myosin RLC. However, a WT-S195D-mediated increase in force was not accompanied by any increase in myofilament calcium sensitivity, as observed in RLC studies. Maximal force generation was not changed in any of ELC-reconstituted fibers when compared to WT-ELC. Two ELC mutants (A57G and E143K) slightly (but significantly) increased Ca^{2+}-sensitivity of force while M173V produced no change. Further studies are needed to reveal whether these subtle changes seen in A57G and E143K-exchanged porcine cardiac preparations could be "rescued" by respective S195D pseudo-phosphorylation mimics. Supported by AHA-12PRE12030412 (WH) and NIH-HL108343 (DSC).

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Structural Defects Induced by Malignant Mutations in the Regulatory Light Chain of Myosin
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The current study is aimed at providing insight into the structural defects underlying the development of malignant phenotypes induced by D166V and R58Q mutations in the regulatory light chain (RLC) of myosin. X-ray diffraction studies were carried out on the freshly skinned papillary muscle fibers from transgenic mutant vs. wild-type mice using the small angle instrument on the BioCAT beamline 18-D at the Advanced Photon Source. The fiber was immersed in relaxing solution and the X-ray measurements were made at two sarcomere lengths (SL), short (~2.3 μm) and long (~2.5 μm). At the short SL, we observed that the R58Q mutation caused a significant increase in the interfilament lattice spacing (d1,0) compared to Tg-WT while no change was observed for the D166V mutation. Interestingly, upon stretch to long SL, WT and R58Q showed a significantly decreased lattice spacing (by ~1.5 nm) while the d1,0 in the D166V myocardium remained the same as measured at short SL. The lack of structural response to stretch observed for D166V may indicate a mutation induced increase in fiber stiffness. In support of this notion, measurements of passive force performed in glycerinated skinned papillary Tg-D166V fibers demonstrated significantly increased levels of passive tension at all points of stretch (10%, 20%, 30% and 40% of fiber length) indicating an elevated resistance to stretch in Tg-D166V compared to Tg-WT fibers. Our results suggest that the mutant-induced structural changes that most likely trigger pathological remodeling of the heart leading to FHC are different for both studied RLC mutations. Supported by AHA-10POST3420009 (PM), NIH- HL071778 (DSC), HL090786 (DSC), P41 GM103622-17 (TI)

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Volume Overload Heart Failure Increases Myocardiac Passive Stiffness in a Mouse Model
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Volume overload (VO) heart failure occurs due to pathologies such as mitral valve regurgitation, infarcts and ventricular septal defects, and leads to characteristic eccentric dilation. Changes in passive myocardial stiffness are not well understood and here we dissected the contribution of extracellular matrix (ECM)-based and titin-based stiffness to overall passive stiffness. Titin is a giant protein that regulates passive tension and hence diastolic function in the myocardium. In heart failure, differential splicing and phosphorylation events can occur that alter the stiffness of this protein and influence hemodynamics. Increased diastolic stiffness due to differential splicing and phosphorylation of titin has been observed in pressure overload hypertrophy; however, there are no published studies investigating whether pure LV VO during compensated heart failure leads to changes in titin based passive stiffness. We studied the role of titin in modulating diastolic function in VO induced by aortocaval fistula (ACF) in the mouse. ACF was induced in three-month-old male C57BL/6 animals and allowed to progress for 4 weeks. At 4 weeks, echocardiography confirmed the presence of eccentric dilation and decreased systolic function as expected. Tissues mechanics indicated an increase in titin, which correlated with a significant decrease in N2BA/N2B ratio. ECM based passive stiffness was unchanged in VO. In summary, VO causes an increase in titin based passive stiffness.